part of the equation, and their precise measurement might be necessary to understand the quantitative relationships that relate synaptic inputs to spiking output. Temporal variability is another big part of the equation: on a short time scale, for example, summation between synaptic inputs coming from different interneurons may vary considerably depending on the relative timing of firing of these neurons, and this would strongly modulate the effect of each synaptic input on the activity of the motorneuron.

The new study by Norris *et al.* [8] is quite enlightening for those who wish to understand the quantitative rules of regulation of biophysical properties that underlie the stability of function of neuronal networks. First, it clearly demonstrates the diversity of solutions in synaptic parameters that produce adequate functional output in a simple neuronal network. In this respect, this study emphasizes once again the very high degree of flexibility that is present in neuronal networks, in this particular case not at the wiring level but at the biophysical level. Secondly, it shows that knowing the strengths of *all* of the synaptic inputs to a neuron is not sufficient to predict its behavior in the absence of knowing a good deal about its intrinsic membrane properties. However, in their new work Norris *et al.* [8] already flirt with the upper limits of the number of electrophysiological parameters that can be measured in the same preparation, and obtaining the complete picture of the parameter space may have to await the advent of new experimental techniques. In conclusion, this work suggests that general rules most certainly exist that allow the function of a given system to be fairly stable across individuals, but the individual-to-individual dynamic adaptations of these rules and the vast number of biophysical parameters involved may often prevent us from deciphering them.

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Animal Communication: Flies' Ears Are Tuned In

Male fruit flies sing to females with quiet, close-range wing vibrations. A new study has found that the flies' antennal ears show active tuning to the species-specific frequencies of songs.

Elina Immonen and Michael G. Ritchie

Understanding the sensory processes involved in animal communication is vital to studies of mate recognition, sexual selection and speciation [1,2]. Studies of communication in the fruitfly *Drosophila melanogaster*, the main signals of which are the male courtship song and pheromones, have contributed greatly to our knowledge of the evolution and genetic control of sexual communication. We know much about the production of song and both the production and perception of pheromones, but more modest progress has been made understanding the sensory perception of acoustic signals [3]. The demonstration by Riabinina *et al.* [4], reported in this issue of *Current Biology*, that *Drosophila* 'ears' are actively tuned to the acoustic frequencies of the species-specific sound pulses promises to open up a new avenue of research into the evolution and coevolution of sexual signalling in fruit flies.

Male flies serenade females with song produced by wing vibration, and females hear this song by detecting the resultant waves of air particle displacement [5]. *Drosophila* ears are modified antennae, which consist of two functional units, feather-like hairs (arista) attached to a segment called the funiculus. Together these form the sound receiver module, which rotates back and forth in response to the moving air particles. The funiculus is joined to the second segment, the pedicellus, which harbours the hearing neurons within a structure called Johnston's Organ [6].

The fly ear works as a non-linear mechanical oscillator, which is particularly suitable for near-field song detection [7]. Fly song is only effective over a very short distance, and male flies only sing when close to a female [5]. Even at a distance of only a few millimetres the song is not very loud. How do the females detect it? Active mechanical feedback from mechanotransducer channels in the membranes of Johnston's Organ neurons augments the sound-induced antennal movement

in an intensity dependent manner, thereby enhancing the auditory sensitivity for low sound intensities [8]. The active amplification also shifts the tuning of the ear to a lower frequency range [7].

Does this level-dependent tuning adjust the hearing to species-specific song, allowing differentiation between conspecific and heterospecific males? Riabinina *et al.* [4] tackled this question by exploring the antennal tuning of seven different species from the *melanogaster* group of *Drosophila.* They focused on one of the song components, the principal frequency within the short sound pulses, to test whether the antennal ears of females of different species are tuned to frequencies that fall within the frequency range of conspecific males' pulse song. Like the hair cells in our ears, the fly arista twitches spontaneously in the absence of sound. Laser Doppler vibrometry was used to measure these free fluctuations and determine the receivers' best frequencies for sound stimulus detection. These best frequencies showed substantial differences between species, ranging from around 150 Hz in *D. melanogaster* to nearly 300 Hz in *D. mauritiana*. Interestingly, repeating the experiment using CO_2 anesthetised flies showed a steep increase in the resonant frequency range, thus revealing their passive hearing ranges, which are largely overlapping between the species. This is consistent with the level-dependent transducer model, and shows that fly ears are tuned into particular regions of the lower frequency range, and this tuning is active rather than passive.

In order to show the receiver spectral tuning is indeed for song, the authors examined intra-pulse frequencies from recorded songs and demonstrated a strong correlation with the ears' best frequencies. Further, stimulating the antennae of *D. teissieri* with pulse-like low amplitude sound of an appropriate frequency showed high antennal displacement gains, whereas stimulation with high amplitude sound was less effective or tuned [4].

These findings are an exciting contribution to recent advancements in unravelling the mechanisms of *Drosophila* audition. Similar species-specific neural tuning to conspecific acoustic signals has been demonstrated in a range of species from other invertebrates [9] to primates [10]. However, with the wealth of tools available in *Drosophila* for studying hearing at the molecular, neural and physiological levels, we now have an opportunity to probe further into the mechanisms of species-specific communication. Recently, the neurons specific to hearing song were identified within Johnston's Organ [11,12], which now allows further analyses of their role in detecting song frequencies, as well as other song parameters. Indeed, a study by Yorozu *et al.* [12] also indicates some differentiation between the two sets of hearing-related neurons in their response to different frequencies of pulse-like sound.

The evolutionary implications are also very intriguing. At one level, it is perhaps surprising to find such peripheral sensory tuning in a communication system involving short sound pulses. Early playback studies implied that the frequency of sound in the pulses of *D. melanogaster* song was not important to female choice [13]. Another reason people have not concentrated on the potential importance of intrapulse frequency is more prosaic. One might expect that if frequency was important the pulses would be longer in length to allow females to distinguish song frequency more efficiently (indeed, the experimenters manipulated the relationships of sound pulses into phase to facilitate measuring their frequency). Species of other groups of *Drosophila*, which are known to place importance on pulse frequency, tend to have longer, more polycyclic sound pulses [14,15]. Therefore, it is perhaps something of an enigma why short pulses would evolve if their frequency content were important. Could it be the case that efficient tuning is particularly important *because* the pulses are constrained to be short? Many of the *melanogaster* group species also produce sine song with a more sustained wing waggle, which allows clearer frequency resolution, though it is often a relatively minor component of the acoustic repertoire and the frequency is less variable between species. Temporal parameters of pulse repetition rates, such as the mean and patterns in the variability of interpulse interval, have been better studied in the *melanogaster* species [16,17], as have additional modes of signalling, especially pheromonal communication [18].

Another issue is the familiar concern in communication studies of the relative importance of peripheral tuning versus central processing of signals. Filtering out heterospecific song at a peripheral level will very effectively discriminate against heterospecifics, and central processing can only be done effectively on signals which are not filtered out. Both processes will determine communication effectiveness, but their relative importance is often uncertain. Combined studies of actual behavioural mate choice, for example in response to synthetic song, teamed with studies of sensory physiology are required to disentangle the relative importance of peripheral sensory versus central processing mechanisms in the analysis of mate choice, and these could now be particularly informative with *Drosophila*.

These studies bring a new perspective to our understanding of the sensory mechanisms involved in acoustic communication in fruit flies. Similar breakthroughs have been made recently in the demonstration of unexpectedly complex species-specific frequency tuning of mosquito aristae to harmonics (rather than fundamental frequencies) of their acoustic signals [19]. Now that the mechanism of species-specific hearing in fruit flies is better understood, we can make more progress in identifying the chain of processes involved, including the neural and genetic control of the system. In particular, more progress is required in understanding how the filtered messages from peripheral sensory receptors are processed to allow identification of the signal content, in terms of species identity or signaller quality.

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Invadopodia: RhoC Runs Rings around Cofilin

Tumor cell invadopodia mediate degradation of matrix barriers. A new study now demonstrates that a ring of active RhoC focuses invadopodial protrusion and degradation by regulating cofilin activity.

Stacey M. MacGrath[1] and Anthony J. Koleske[1,2]

Complications from metastasis are the primary cause of breast cancer mortality, making the pathways that regulate this process attractive targets for therapeutic intervention. To penetrate surrounding tissues, cancer cells must invade the basement membrane, a network of extracellular matrix proteins that supports the overlying epithelium. Once they have escaped the tumor, metastasizing cells must migrate through the stroma and degrade the vascular subendothelial basement membrane to gain entry to the bloodstream. In culture, invasive cancer cells cross similar matrix barriers by forming F-actin-rich protrusions called invadopodia [1], which provide localized delivery of matrix metalloproteinases to degrade these barriers. The formation of invadopodia correlates with cell invasiveness.

Invadopodia proceed through several different stages to mature into functional, matrix-degrading structures [2]. First, small clusters of branched F-actin, the actin-nucleation-promoting factors cortactin and N-WASp, cofilin, and the actin-related protein (Arp) 2/3 complex form invadopodia cores. These clusters have two fates: they can either dissociate or become stable invadopodia. Chemotactic stimuli within the tumor stroma, such as epidermal growth factor (EGF), promote new actin synthesis within invadopodia, leading to their stabilization, protrusion and, finally, degradation of the surrounding matrix [2–5]. Once they have emerged, invadopodia elongate through convergent extension of a central bundle of actin filaments. The initial invadopodial protrusion can enlarge to create a larger breach that ultimately allows the cell to penetrate the membrane and invade the surrounding tissue [6].

Tightly focused invadopodial penetration of the basement membrane appears to be a critical first step in invasion. But why the tight focus? Basement membranes are likely the most difficult barriers to breach. Focusing of invadopodia may concentrate matrix metalloproteinase activity. Moreover, the convergent elongation of actin filaments within a concentrated site of protrusion would be expected to produce the maximal unit force for basement membrane penetration. Invadopodia may also act as microsensors, testing the matrix environment to seek out favorable routes of invasion [7]. These factors likely explain why invadopodia must be so narrowly focused.

How is this tight focus maintained? The Ena/VASP family protein Mena localizes to invadopodia and promotes the formation and maturation of these protrusions [8]. By virtue of its ability to promote actin filament elongation, Mena likely supports convergent extension of actin filaments within the invadopodial core. In addition, actin-bundling proteins, such as fascin and T-fimbrin [9], stabilize F-actin bundles within the invadopodial core. Nevertheless, both of these mechanisms likely require a tight initial grouping of nascent elongating actin filaments. The major unresolved question is what corrals the nascent invadopodial protrusion. Now, in a paper in this issue of *Current Biology*, Bravo-Cordero *et al.* [10] reveal a novel mechanism by which the RhoC GTPase focuses actin polymerization within the assembling invadopodium. In so doing, the authors may have solved an important mystery as to the function of the RhoC GTPase in cancer metastasis.

Bravo-Cordero *et al.* [10] initially found that knockdown of the RhoC GTPase in highly metastatic MTLn3 rat breast carcinoma cells reduced their migration through matrix barriers. This finding is consistent with previous work demonstrating that RhoC is upregulated in invasive cancers and that RhoC overexpression can drive melanoma cell metastasis [11].
In contrast to its more famous relatives, RhoA, Rac1, and Cdc42, RhoC is comparatively understudied. Thus, the molecular mechanisms by which RhoC regulates tumor cell invasion and metastasis were unclear.

A major clue to RhoC function came from the analysis of invadopodial